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Short Communication

Escherichia coli O157 in Bovine Feces and Surface Water Streams in a Beef Cattle Farm of Argentina

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) is an important foodborne pathogen, and ruminants are recognized as the main natural reservoir. The purposes of this study were to detect *E. coli* O157 in bovine feces and surface water in a beef cattle farm of Gualeguaychú, Argentina; to characterize the isolates; and to establish the clonal relatedness by pulsed-field gel electrophoresis. Between September 2005 and November 2006, 288 samples of bovine feces and 79 samples of water troughs were studied. *E. coli* O157 was detected by immunomagnetic separation and polymerase chain reaction as screening techniques. The rfb_{O157} gene was detected in 3.8% of the 288 fecal samples and in 17.7% of the 79 water samples. The *stx* gene was detected in all rfb_{O157}-positive fecal samples and in 5.1% of water samples. Eleven *E. coli* O157 strains isolated from bovine fecal samples and eight from water samples were characterized. The most frequent *stx* genotype identified was stx_1 and $stx_{2c(vh\text{-}a)}$. Twelve (63.2%) strains harbored $fliC_{H7}$, *eae*, and *ehx*A genes. Using pulsed-field gel electrophoresis with the enzyme *Xba*I, a total of eight patterns with at least 72.1% similarity were identified among the 19 strains. The patterns of 15 strains were grouped into four clusters: two of them included only bovine strains and the other two only aquatic strains. No genetic correlation was established between the bovine and water STEC strains detected. The prevalence of STEC O157:H7 established in the herd studied was higher than that previously reported for Argentine grazed cattle.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are zoonotic agents that are widely distributed around the world and are important foodborne pathogens. The prototype strain is *E. coli* O157:H7. Cattle are considered the main animal reservoir for STEC O157:H7, and the first isolates in cattle were described in Argentine calves in 1977 (Caprioli *et al.*, 2005). STEC infection can cause bloody diarrhea and hemolytic uremic syndrome (HUS). Argentina has the highest HUS rate globally—13.9/100,000 children under age 5—and *E. coli* O157:H7 has been related to almost 75% of the cases (Rivas *et al.*, 2006). The purposes of this study were to detect *E. coli* O157, especially serotype O157:H7, in bovine feces and water in a beef cattle farm of Gualeguaychú, Argentina; to characterize the isolates; and to establish the clonal relatedness by pulsed-field gel electrophoresis (PFGE).

Materials and Methods

Between September 2005 and November 2006, 288 swabs of rectal feces from healthy animals (Rice *et al.*, 2003) and 79 water samples (Spira and Ahmed, 1981) were collected. Sample size was calculated using the Epi Info software (version 6.0), taking into account an estimated frequency of 10% and a precision of 1.5% at 95% confidence level. The estimated prevalence was based on a 0.5% prevalence previously reported (Meichtri *et al.*, 2004), which was multiplied by a factor of 20 because of an increase in sensitivity resulting from the use of the immunocapture system. Rectal swabs were placed in 100 mL of modified EC broth (Biokar Diagnostics, Beauvois, France) with 20 mg/L of sodium novobiocin ($_mEC + N$; Sigma Chemical, St. Louis, MO). The water samples were taken with modified Moore swabs from two streams, one inside the study area (200 acres), accessible for the animals, and the other downhill out of the field. The swabs were placed in flasks with 100 mL of $_mEC + N$. Enrichment broths were incubated for 24 h at 42°C and processed by immunomagnetic separation (Dynal, Compiàgne, France); the immunoconcentrates were streaked on two media: O157:H7 ID™ (bioMérieux, Marcy l'Etoile, France), and MacConkey sorbitol agar (Biokar) supplemented with 2.5 mg/L of potassium tellurite and 0.05 mg/L of cefixime (bioMérieux). After incubation, confluent growth zone and

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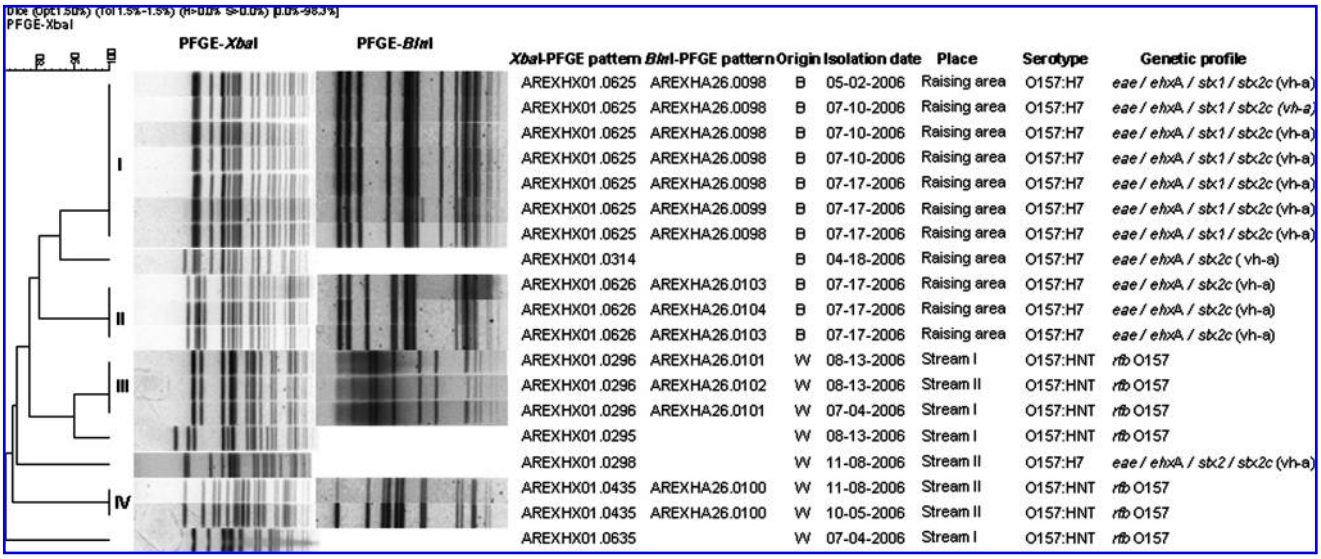
individual colonies were screened for stx_1, stx_2, and rfb_{O157} genes by multiplex polymerase chain reaction (PCR) (Leotta *et al.*, 2005).

The isolates were characterized by standard biochemical tests (Ewing, 1986) and genotypic techniques (Leotta *et al.*, 2008). The antimicrobial susceptibility was carried out by the Kirby–Bauer method following the recommendations of the Clinical and Laboratory Standards Institute. *Xba*I-PFGE was performed according to the Centers for Disease Control and Prevention protocol (CDC, 2007). *Bln*I (Promega, Madison, WI) was used as second enzyme.

Results and Discussion

Eleven (3.8%) out of the 288 fecal samples were rfb_{O157} positive. Two samples were detected in autumn and nine in winter. STEC strains were isolated from all rfb_{O157}-positive samples. Four (36%) of them harbored stx_2 genes, and seven (64%) carried stx_1 and stx_2 sequences, simultaneously (Fig. 1). All strains were β-D-glucuronidase negative, cytotoxic on Vero cells, showed enterohemolysis production, and were susceptible to ampicillin, ampicillin-sulbactam, piperacillin, cephalothin, cefoxitin, cefuroxime, cefotaxime, ceftazidime, nalidixic acid, ciprofloxacin, trimethoprim–sulfamethoxazole, nitrofurantoin, gentamicin, chloramphenicol, fosfomycin, and tetracycline. The PCR-restriction fragment length polymorphism genotyping method showed that seven strains harbored stx_1 and $stx_{2c(vh\text{-}a)}$ genes and four carried only $stx_{2c(vh\text{-}a)}$ sequence. All strains were positive for $fliC_{H7}$, *eae*, and *ehx*A genes. Fourteen (17.7%) out of the 79 water samples were rfb_{O157} positive. Three samples were detected in spring, four in summer, one in autumn, and six in winter. However, *E. coli* O157 strains were isolated from eight (10.1%) water samples. Seven strains had none of the genes for virulence factors. The STEC strain isolated from a water sample showed the same phenotypic characteristics as the bovine isolates and harbored stx_2 and $stx_{2c(vh\text{-}a)}$, $fliC_{H7}$, *eae*, and *ehx*A genes. *Xba*I-PFGE was able to subtype the 19 *E. coli* O157 strains, generating eight different patterns with 16–21 discernible fragments, ranging approximately from 36 to 582 kb in molecular weight. Fifteen strains with indistinguishable profiles were grouped into four clusters, and four strains showed unrelated *Xba*I-PFGE patterns (Fig. 1). Cluster I (AREXHX01.0625) included seven toxigenic bovine strains isolated in May and July 2006. Six of them presented the same *Bln*I-PFGE pattern, and the remaining strain differed in only one band, with 97.4% similarity. Cluster II (AREXHX01.0626) grouped three toxigenic bovine strains, isolated at the same sampling date (July 17, 2006), as some of the strains grouped in cluster I. They were differentiated into two *Bln*I-PFGE profiles, with only one band difference and 97.3% similarity. Cluster III (AREXHX01.0296) included three nontoxigenic strains isolated from water. Two of them were isolated in August 2006, in two different streams. One strain of this cluster differed in the *Bln*I-PFGE profile, with only one band difference and 96% similarity. It was ampicillin resistant. Cluster IV (AREXHX01.0435) included two nontoxigenic water strains isolated in the same stream in October and November 2006. These strains were indistinguishable by *Bln*I-PFGE.

A previous report from Argentina confirmed that calves harbored STEC O157:H7, with a prevalence of 0.5% (Meichtri *et al.*, 2004). In this study, the prevalence of *E. coli* O157:H7 in fecal samples of grazed cattle was 3.8%. These isolates were recovered during autumn and winter in 2006. Both precipitation (240.5 mm) and temperature (10.6–19.8°C) in May, June, and July 2006 were higher than normal (Argentine National Weather Service). In water samples, *E. coli* O157 could only be isolated from 57% of PCR-positive samples but only one isolate was toxigenic. This strain harbored stx_2 and $stx_{2c(vh\text{-}a)}$ genes, which is the prevalent genotype in bloody diarrhea and HUS cases (Rivas *et al.*, 2006). It presented the AREXHX01.0298 profile not previously included in the *E. coli* O157 Argentine Database. However, Shiga toxin-negative *E. coli* O157 strains detected in water could be able to acquire *stx* genes as described by Wetzel and LeJeune (2007). The eight strains recovered from water showed five different *Xba*I



XbaI-PFGE pattern	BlnI-PFGE pattern	Origin	Isolation date	Place	Serotype	Genetic profile
AREXHX01.0625	AREXHA26.0098	B	05-02-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0625	AREXHA26.0098	B	07-10-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c (vh-a)*
AREXHX01.0625	AREXHA26.0098	B	07-10-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0625	AREXHA26.0098	B	07-10-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0625	AREXHA26.0098	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0625	AREXHA26.0099	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0625	AREXHA26.0098	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx1 / stx2c* (vh-a)
AREXHX01.0314		B	04-18-2006	Raising area	O157:H7	*eae / ehxA / stx2c* (vh-a)
AREXHX01.0626	AREXHA26.0103	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx2c* (vh-a)
AREXHX01.0626	AREXHA26.0104	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx2c* (vh-a)
AREXHX01.0626	AREXHA26.0103	B	07-17-2006	Raising area	O157:H7	*eae / ehxA / stx2c* (vh-a)
AREXHX01.0296	AREXHA26.0101	W	08-13-2006	Stream I	O157:HNT	*rfb* O157
AREXHX01.0296	AREXHA26.0102	W	08-13-2006	Stream II	O157:HNT	*rfb* O157
AREXHX01.0296	AREXHA26.0101	W	07-04-2006	Stream I	O157:HNT	*rfb* O157
AREXHX01.0295		W	08-13-2006	Stream I	O157:HNT	*rfb* O157
AREXHX01.0298		W	11-08-2006	Stream II	O157:H7	*eae / ehxA / stx2 / stx2c* (vh-a)
AREXHX01.0435	AREXHA26.0100	W	11-08-2006	Stream II	O157:HNT	*rfb* O157
AREXHX01.0435	AREXHA26.0100	W	10-05-2006	Stream II	O157:HNT	*rfb* O157
AREXHX01.0635		W	07-04-2006	Stream I	O157:HNT	*rfb* O157

FIG. 1. Phenotypic and genotypic characterization and clonal relatedness, into 4 clusters, of 19 *Escherichia coli* O157 strains isolated from cattle and water in a beef cattle farm, Gualeguaychú, Argentina, 2005–2006.

profiles, whereas the 11 bovine strains showed only three *Xba*I profiles. This would indicate that there is more diversity in the aquatic environment.

In conclusion, in the herd studied we found (a) a higher prevalence of STEC O157:H7 than that previously reported for Argentine grazed cattle, (b) the toxigenic strain recovered from water was genetically different from the toxigenic animal strains, and (c) during the study period, strains from both sources, animal and environment, showed different virulence profiles. However, other studies should be conducted to better understand the risk of STEC transmission in Argentina, considering bovine and environmental reservoirs or both.

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Disclosure Statement

No competing financial interests exist.

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